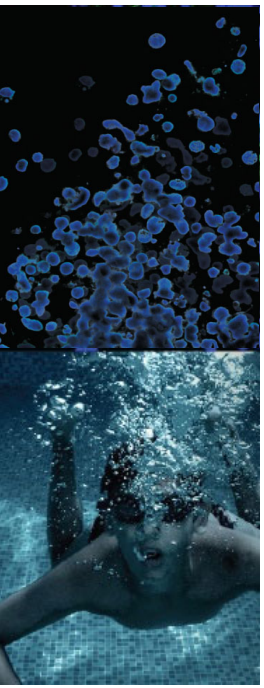
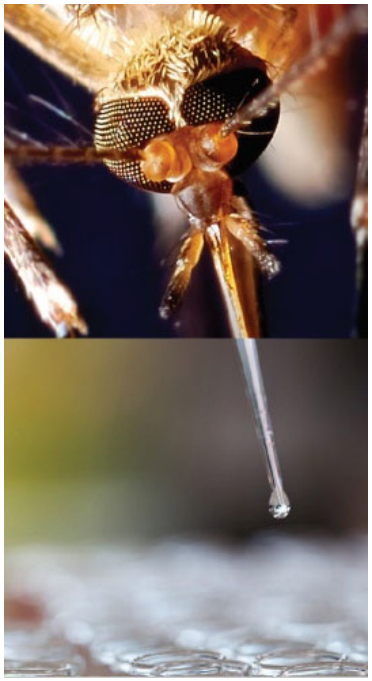
Expanded Primary Hepatocytes: Achieve More Predictive Toxicity Studies

Kevin Grady, B.S.
Senior Product Line Business Manager, ATCC

Credible Leads to Incredible™





About ATCC

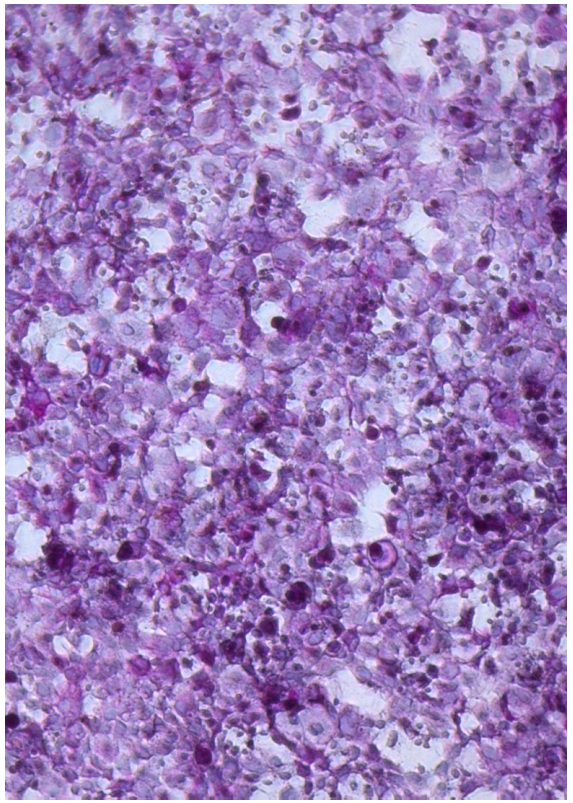
- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World's largest, most diverse biological materials and information resource for microbes – the "*gold standard*"
- Innovative R&D company featuring gene editing, microbiome, NGS, advanced models
- cGMP biorepository

- Partner with government, industry, and academia
- Leading global supplier of authenticated cell lines, viral and microbial standards
- Sales and distribution in 150 countries, 18 international distributors
- Talented team of 450+ employees, over one-third with advanced degrees

ATCC®

Agenda

- Heptocyte models
- The upcyte® solution
- upcyte® Hepatocyte characterization
- Applications
- Summary



Problems of current hepatocyte models

Attribute	Continuous Cell Lines	Stem Cell-derived Cells	Primary Hepatocytes
Physiology	Abnormal - tumor	Normal	Normal
Maturity	Mature - adult	Immature - embryonic	Mature - adult
Stability	Dedifferentiation	Stable	Dedifferentiation
Donor availability	None	Limited	Multiple
Availability per donor	Unlimited	Unlimited	Limited
Lot-to-lot variability	Small	Small	Large
Predictability	Low	Low	High

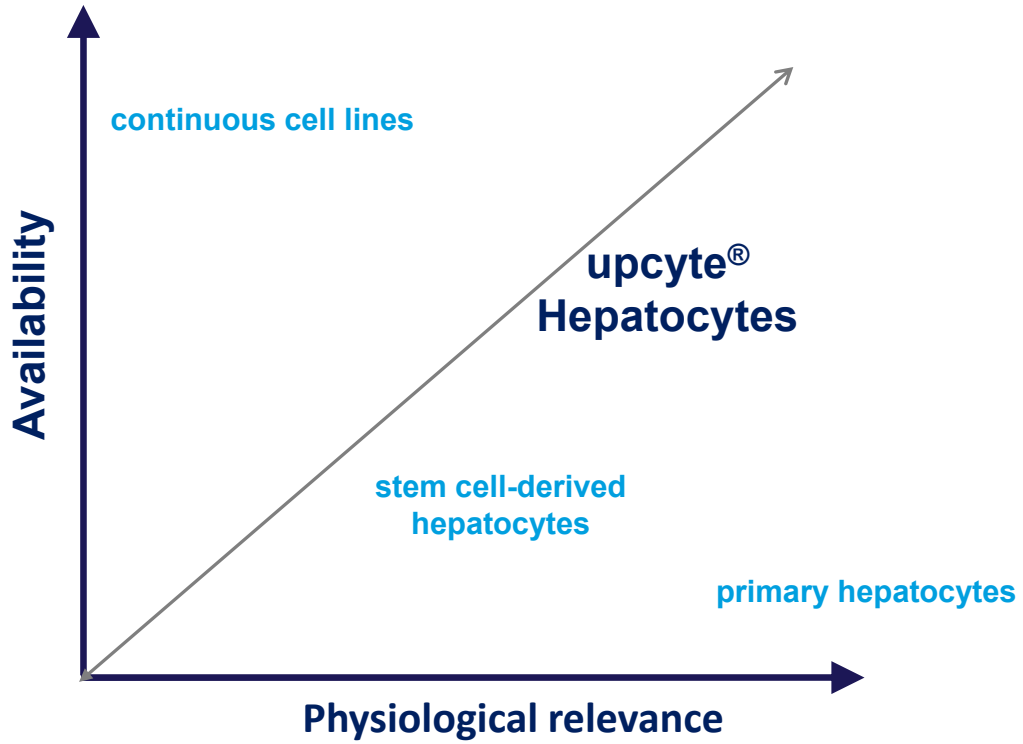
ATCC

The upcyte® Hepatocyte solution

Attribute	Continuous Cell Lines	Stem Cell-derived Cells	Primary Hepatocytes	upcyte® Hepatocytes
Physiology	Abnormal - tumor	Normal	Normal	**Normal**
Maturity	Mature - adult	Immature - embryonic	Mature - adult	**Mature - adult**
Stability	Dedifferentiation	Stable	Dedifferentiation	**Stable**
Donor availability	None	Limited	Multiple	**Multiple**
Availability per donor	Unlimited	Unlimited	Limited	**Unlimited**
Lot-to-lot variability	Small	Small	Large	**Small**
Predictability	Low	Low	High	**High**

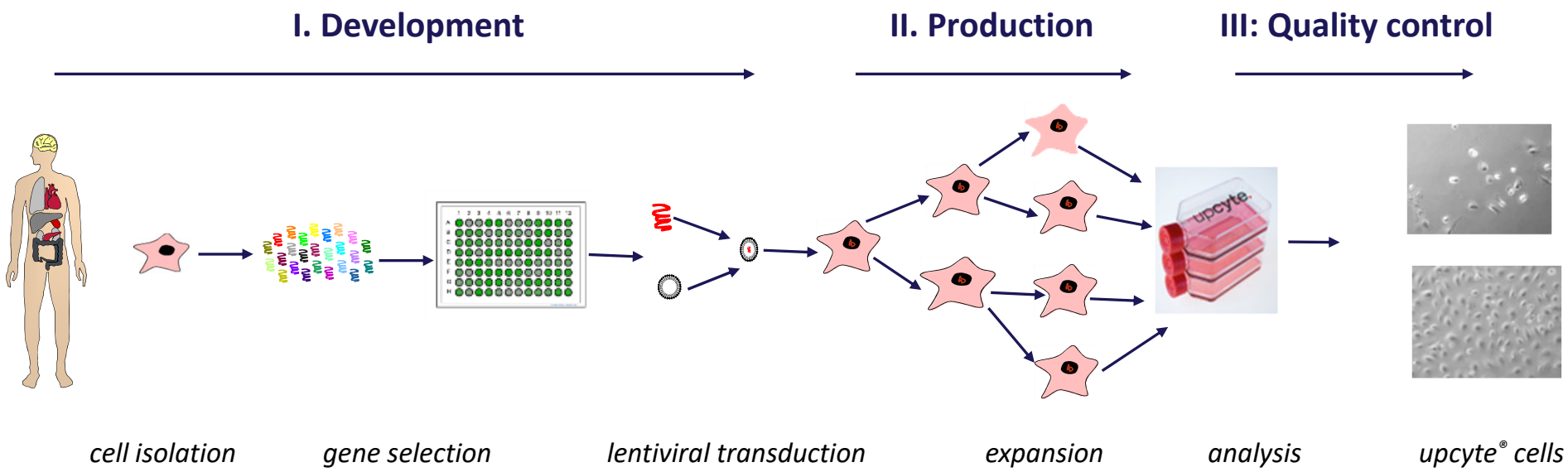
ATCC®

The upcyte® solution – a better cellular model

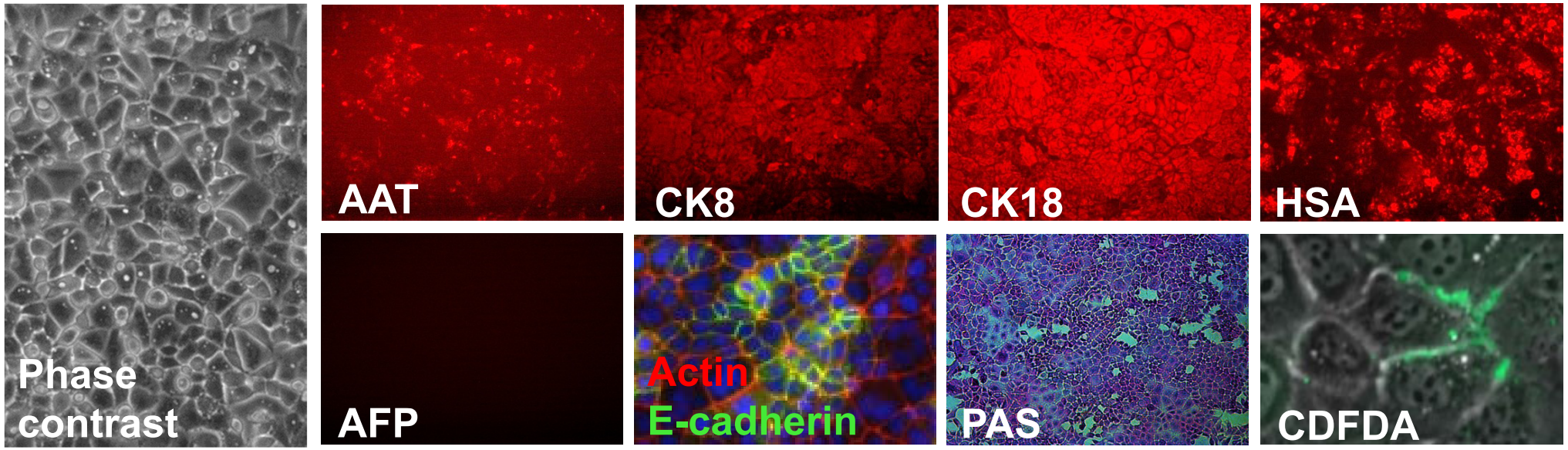


upcyte® technology and production

Proprietary manufacturing process



upcyte® Hepatocytes display adult phenotype



upcyte® hepatocytes express cytokeratin 8 (CK8), cytokeratin 18 (CK18), human serum albumin (HSA), α-anti-trypsin (AAT), but lack embryonic markers such as α-fetoprotein (AFP). The cells further expressed E-cadherin and demonstrated marked capability for glycogen storage (PAS staining) and bile secretion (CDFDA staining).

Data supplied by upcyte® technologies.

upcyte® Hepatocytes maintain metabolic activity

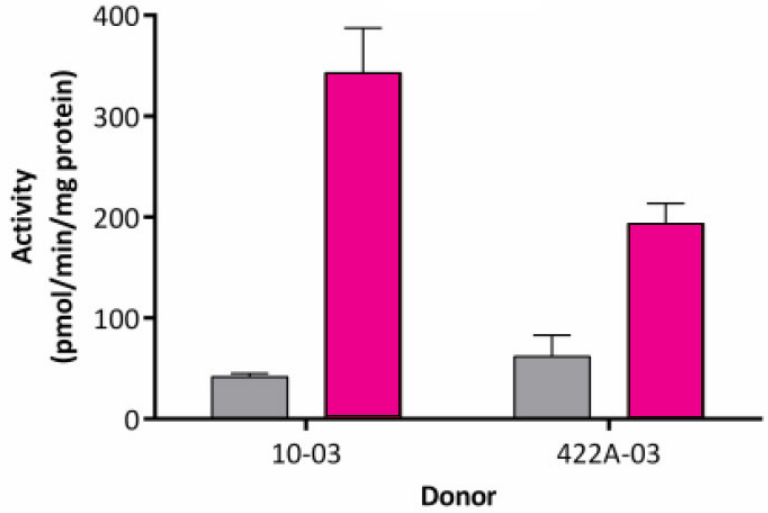
Phase I Activity [pmol/min/mg]	Donor 10-03	Donor 151-03	Donor 422A-03	Donor 653-03
CYP1A2	3.3 ± 0.4	0.7 ± 1.4	2.3 ± 0.1	17.1 ± 0.5
CYP2B6	40.3 ± 6.5	71.1 ± 11.3	33.6 ± 11.4	68.4 ± 18.4
CYP2C9	91.8 ± 5.5	29.1 ± 21.4	4.8 ± 3.1	16.2 ± 0.9
CYP3A4	21.4 ± 9.6	77.8 ± 22.6	42.9 ± 6.3	178.3 ± 17.0

Phase II activity [pmol/min/mg]	upcyte® Hepatocytes	Primary Hepatocytes
SULT (Hydroxycoumarin)	6-16	5-98
UGT (Hydroxycoumarin)	32-345	15-496
GST (CDNB)	15-88	21-35

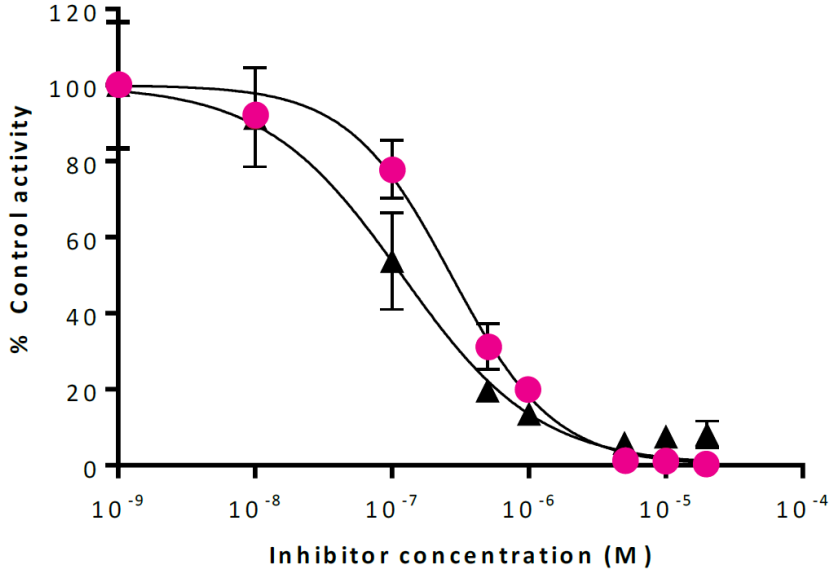
Data supplied by upcyte® technologies.

upcyte® Hepatocytes – metabolism

Induction of CYP3A4 (by rifampicin)
Donors 10-03 and 422A-03

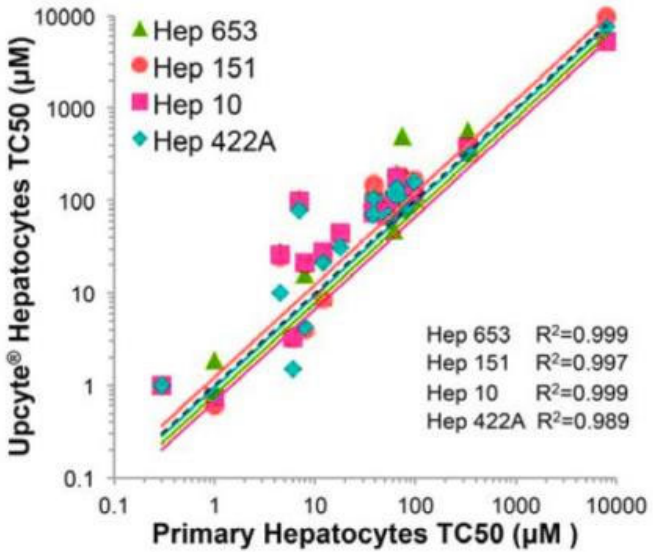


Inhibition of CYP3A4 (by ketoconazole)
Donor 653-03



Data supplied by upcyte® technologies.

TC_{50} correlates between upcyte® and primary hepatocytes



Levy *et al. Nature Biotechnol* 33(12): 1264-71, 2015.

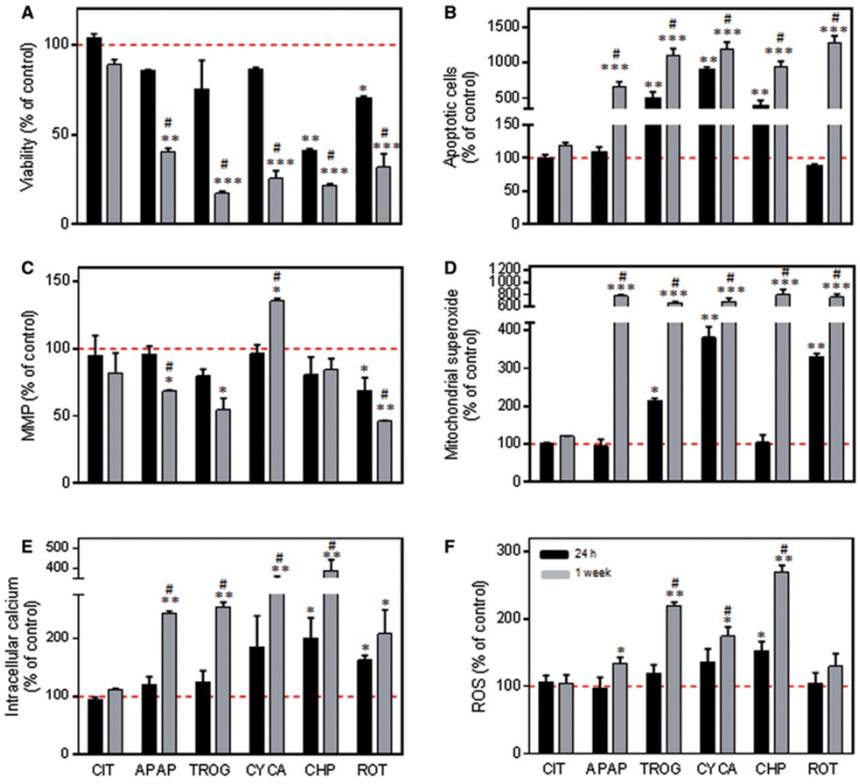
Comparison of the TC_{50} of 18 model compounds in upcyte® Hepatocytes and primary human hepatocytes. Toxicity was measured using the MTS assay. All donors showed an R^2 correlation of 0.99 (n=3).

Cytotoxicity – acute and repeated-dose studies

Employed compounds:

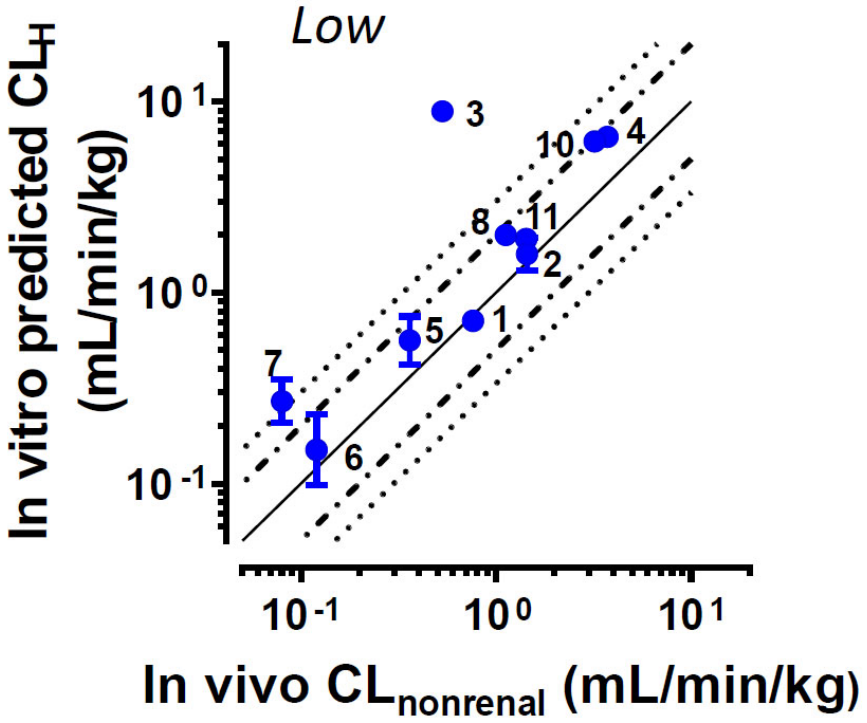
- CIT: Sodium citrate (1-2 mM)
- APAP: Acetaminophen (0.5-2 mM)
- TROG: Troglitazone (50-100 µM)
- CYC(A): Cyclosporin A (20-50 µM)
- CHP: Cumene hydroperoxide (100-500 µM)
- ROT: Rotenone (0.05-1 µM)

upcyte® Hepatocytes were exposed to test compounds for 24 h or 1 week. **(A)** viability, **(B)** apoptosis, **(C)** changes in mitochondrial membrane potential (MMP), **(D)** production of mitochondrial superoxide, **(E)** ROS **(F)** intracellular Ca^{2+} levels using HCS.



Tolosa, *et al. Toxicol Sci* 125 (1): 214-29, 2016.

Clearance prediction



Schaefer, *et al. Drug Metab Dispos* 44(3): 435-44, 2016.

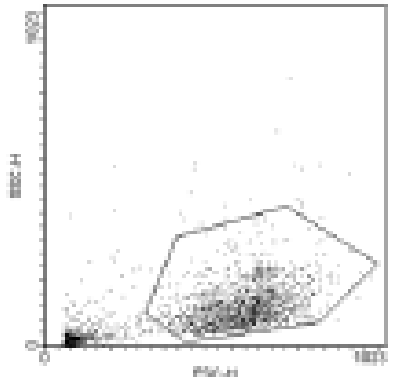
The reference drug set:

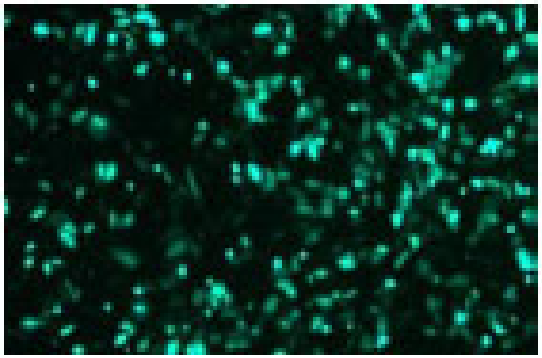
- Alprazolam (1)
- Prednisolone (2)
- Diazepam (3)
- Voriconazole (4)
- Tolbutamide (5)
- Meloxicam (6)
- Warfarin (7)
- Glimepiride (8)
- Riluzole (10)
- Oxazepam (11)

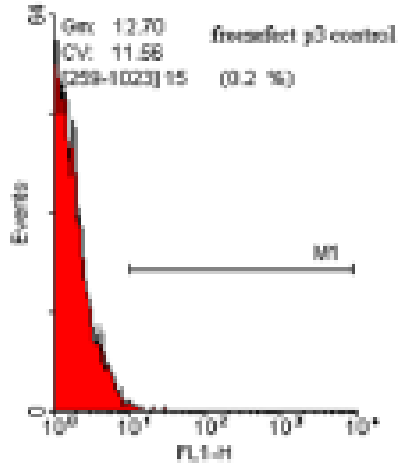
Good correlation between predicted CL_H and observed in vivo CL values was observed for the subset of low CL drugs (shown here). **CL_H for 73%** (8 of 11 compounds) were predicted **within twofold** of in vivo $CL_{nonrenal}$.

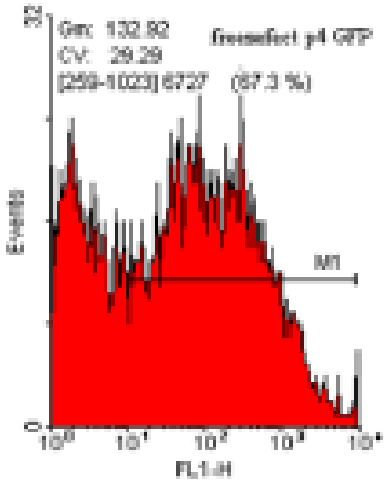
Transfection – transfection with a GFP construct

Transfection mediated using nucleofection.







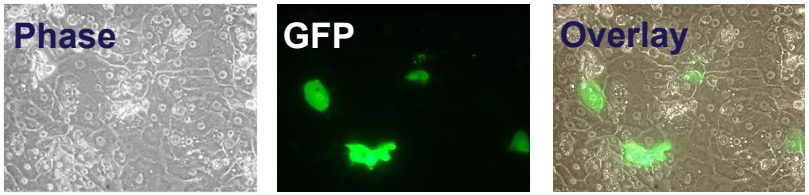


GFP transfected, < 67% efficiency acheived.

Data supplied by upcyte® technologies.

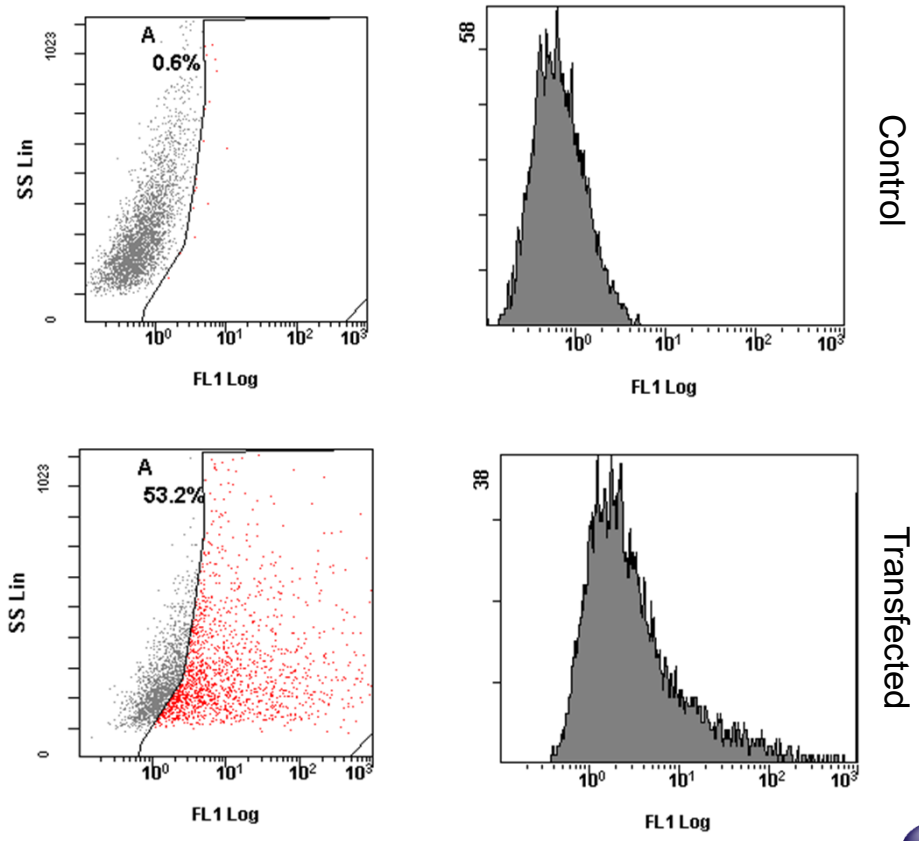
Transfection – lipid delivery system

Primary hepatocytes: < 5% positive cells



upcyte® Hepatocytes:
Up to approximately 50% transfection efficiency possible as demonstrated by GFP expression and flow cytometry.

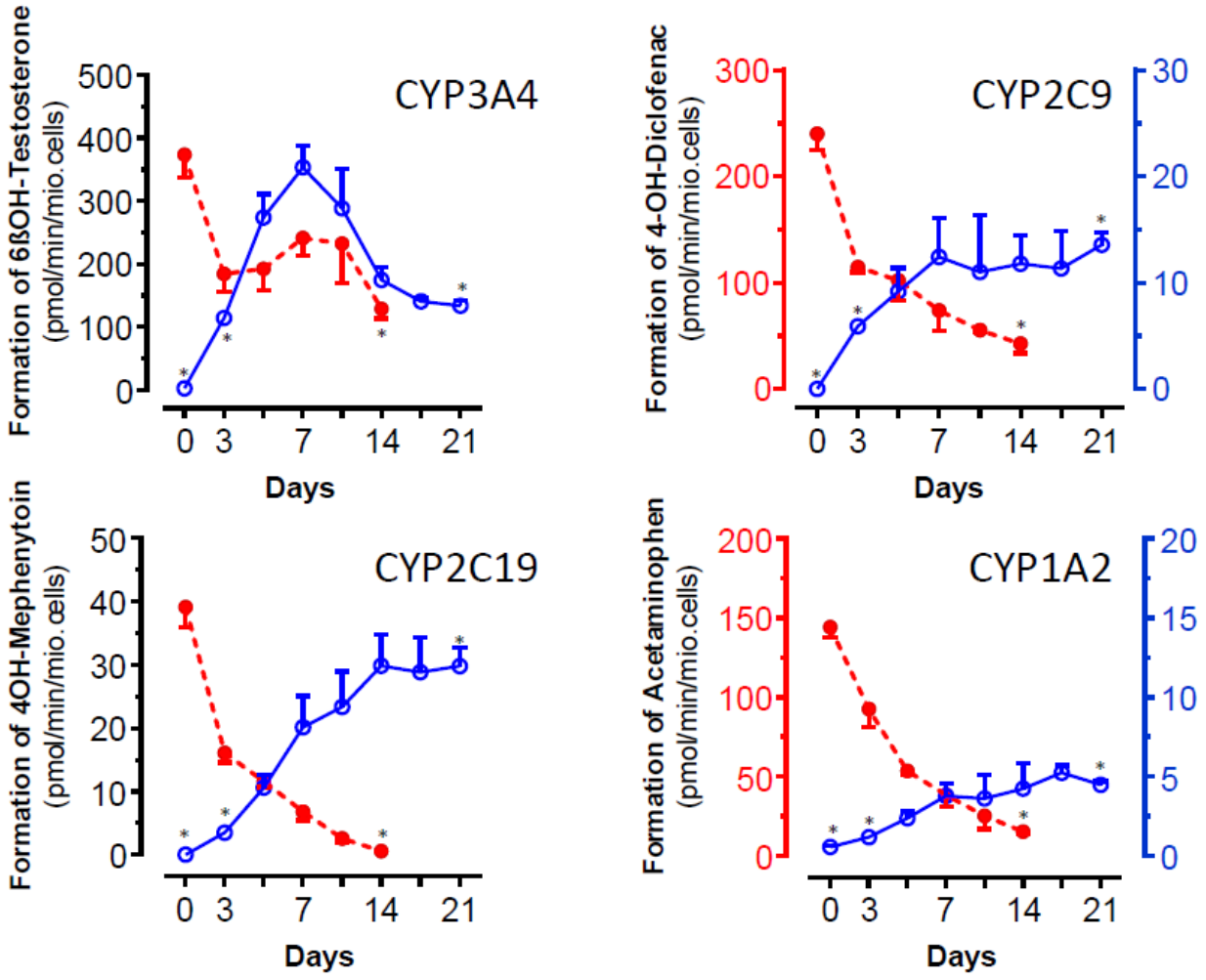
upcyte® Hepatocytes



40-50% positive cells

Data supplied by upcyte® technologies.

upcyte® Hepatocytes – long term cultures



upcyte hepatocytes
primary hepatocytes

Long term culture offers new possibilities

upcyte hepatocytes
primary hepatocytes

Data supplied by upcyte® technologies.

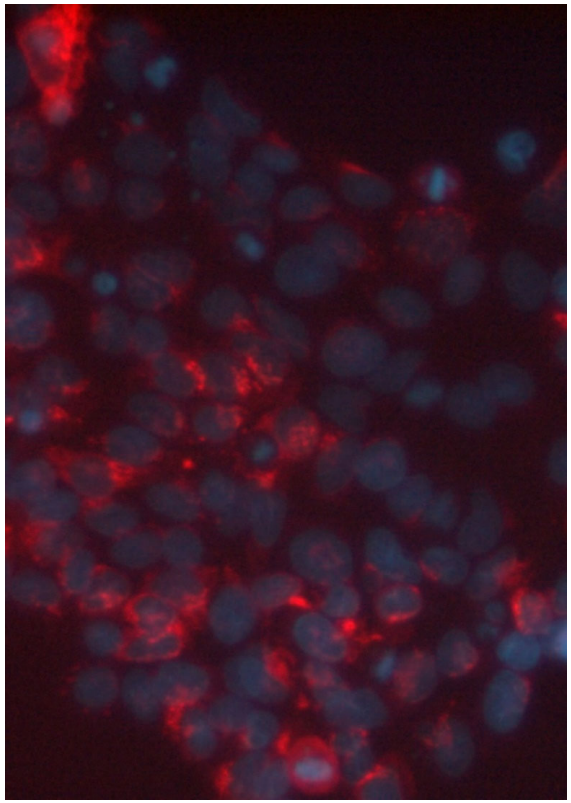
Product format

upcyte® Hepatocytes (ATCC® ACS-9000™)

5 x10^6 cells per vial

Cells tested for:

- Cell morphology
- > 70% viable recovery
- > 90 % plating efficiency
- Markers: CK8+, CK18+; HSA+, AAT+ (α-1-antitrypsin), AFP- (α-fetoprotein)
- Capacity for glycogen storage (PAS staining)
- Basal and inducible CYP activities (Phase I)



Product format

upcyte® Hepatocyte Performance Media Kit (ATCC® ACS-9005™)

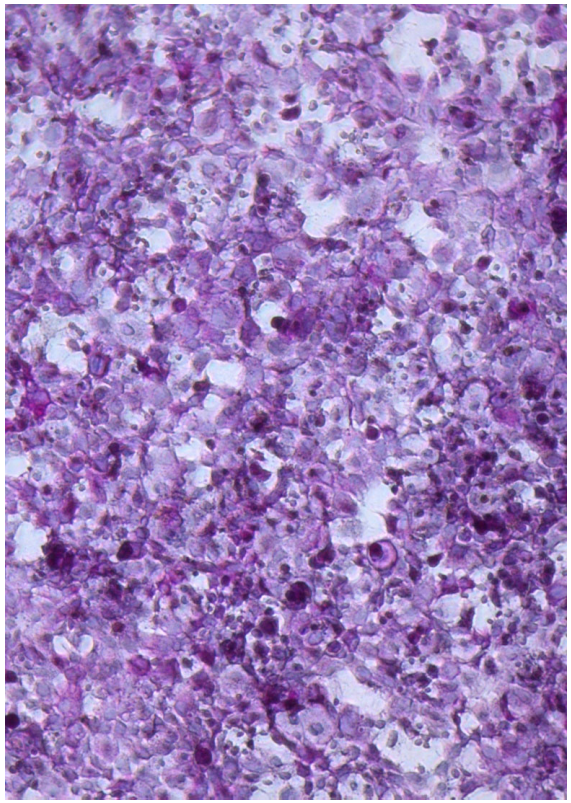
A multi-component kit:

Hepatocyte Performance Medium (ATCC® ACS-9001™; 500 ml) stored at 2-8°C

Hepatocyte Performance Medium Supplements, (ATCC® ACS-9002™) stored at -20°C

- Supplement A (proprietary formulation, ATCC® ACS-9003™; 5ml)
- L-glutamine, (ATCC® ACS-9004™; 5ml)

- Kit components are tested for sterility, mycoplasma, and pH
- Complete medium is tested for growth performance
  - Typical morphology
  - Adherence
  - Growth rate
- Once supplemented, complete medium is stable for 1 month at 2-8°C



Summary – the upcyte® solution

Primary cell features

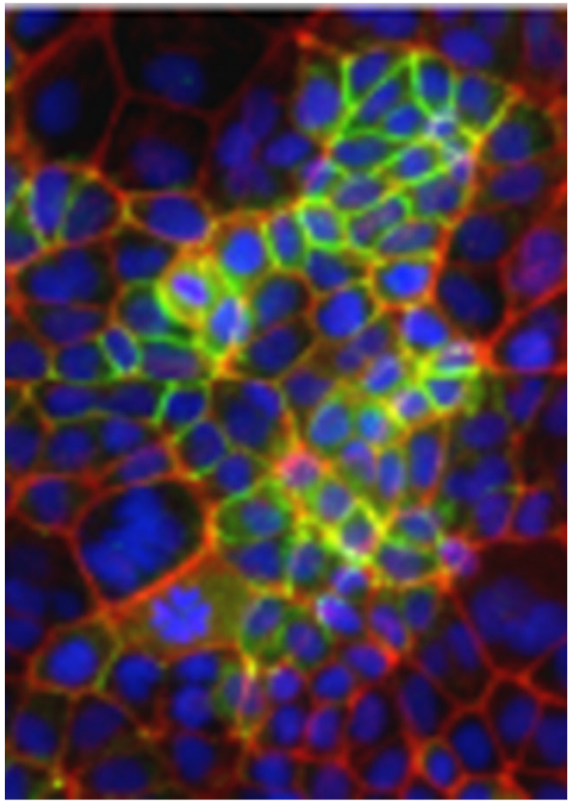
- Generated from healthy human adult cells
- Karyotypically stable
- Physiologically relevant profile
- Cell type-specific phenotype

Extensive availability

- Up to 3000 vials from a single donor
- Supply for screening applications

Flexible use

- 2D & 3D
- Co-culture with other cell types
- Long-term cultures



ATCC®

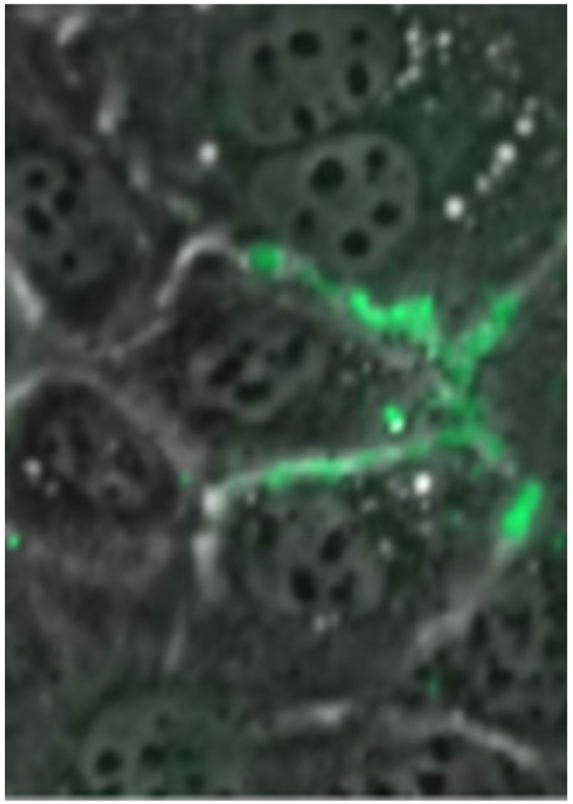
Summary – the upcyte® solution

Easy & safe handling

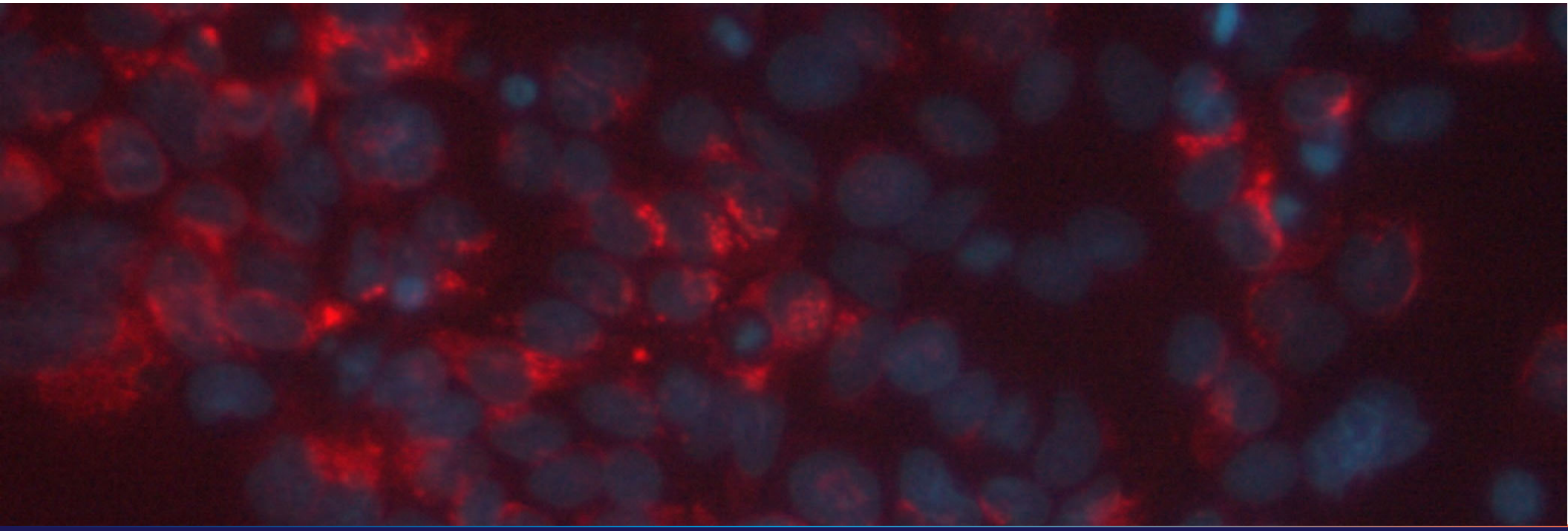
- Quality controlled cells
- Detailed information on cell type specifications
- Standardized procedures for use
- Optimized media

Wide range of applications

- Basic R&D
- Pharmaceutical preclinical development
- ADMET, viral infections



ATCC®



Thank you for your attention. Questions?

ATCC®

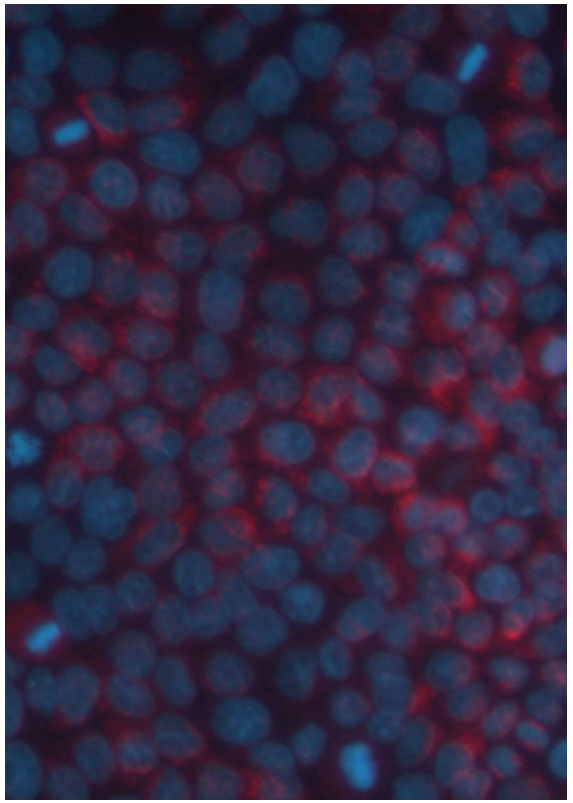
Cultivating collaboration to support global health

Visit **www.atcc.org/expandedhepatocytes** for more information

Visit us at **SOT 59th Annual Meeting & TOXEXPO**,

- March 15-19, 2020, Anaheim, CA
- **Booth #463**
- **Exhibitor-hosted Session:** Immortalized Hepatocytes from ATCC with Full Functionality and Unlimited Availability

www.atcc.org/webinars



ATCC